


In vitro microtubule-binding assays

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 An abbreviated version of this protocol was published in BMC Biology in Sep 2019

CCDC74A/B are K-fiber crosslinkers required for chromosomal alignment

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Detailed protocol

- 1、Remove the microtubule protein from the -80°C refrigerator and dissolve on ice.
- 2、Mix the appropriate amount of microtubule protein with the BRB80 solution (100 mM PIPES, 1 mM MgSO₄, 2 mM EGTA, 1 mM GTP, pH 6.8) and place on ice for 5 minutes.
- 3、Then transfer the reaction system to a metal bath at 37°C and incubate for 30 minutes.
- 4、Stop the reaction with BRB80 buffer (20 uM).
- 5、The polymerized microtubules were pelleted by centrifugation at 45 000 rpm for 45 min at 37 °C.
- 6、Remove the supernatant and resuspend the precipitation (polymerized microtubules) with BRB80 buffer (20 uM).
- 7、GST-labeled proteins are expressed in the E. coli BL21 strain for 16 hours.
- 8、E. coli collection is re-suspended and lysed with GST lysis buffer (0.5% Tween-20, 20 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 5 mM EGTA, pH 7.5).
- 9、Enrich the GST label protein with Glutathione Sepharose 4B beads.
- 9、Wash the beads with GST lysis buffer 3 times and then wash the beads with BRB80 buffer 3 times.
- 10、Incubated the GST beads with polymerized microtubules for 30 minutes at 37 °C.
- 11、Wash the beads 5 times with BRB80 buffer and then added 60 ul 1 × loading buffer, boil the sample at 100°C for 8 minutes to prepare the sample.
- 12、The sample is analyzed by SDS-PAGE followed by Western Blot.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Chen, J. and Teng, J. (2021). In vitro microtubule-binding assays. Bio-protocol Preprint. bio-protocol.org/preprint1156.
2. Zhou, H., Zheng, T., Wang, T., Li, Q., Wang, F., Liang, X., Chen, J. and Teng, J. (2019). CCDC74A/B are K-fiber crosslinkers required for chromosomal alignment. BMC Biology 17. DOI: [10.1186/s12915-019-0694-9](https://doi.org/10.1186/s12915-019-0694-9)

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